

Comparative Study of Interleukin- 6 (Il-6) and Tumour Necrosis Factor Alpha (Tnf-A) Levels in Prostate Cancer and Benign Prostatic Hyperplasia Subjects

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Abstract: Prostate cancer (PC) is an age-related malignancy that is very common in adult males with a very high morbidity and mortality rate worldwide. Chronic inflammation is thought to play a role in carcinogenesis. Pro-inflammatory cytokines, such as interleukin-6 (IL-6) and tissue necrosis factor- α (TNF- α), have been implicated in the pathogenesis of prostate cancer (PC). Its diagnosis remains a challenge even with a combination of methods. Serum prostate specific antigen (Serum-PSA) is the universally accepted biomarker for PC detection. However, it is neither specific nor confirmatory. The aim of this study was to compare IL-6 and TNF- α levels in PC and benign prostatic hyperplasia (BPH) subjects. This cross-sectional, descriptive, non-random study involved 60 histologically diagnosed PC and 20 BPH subjects attending the Urology Clinic of the University of Port Harcourt Teaching Hospital (UPTH). The Enzyme Linked Immunosorbent Assay (ELISA) technique was used to assay the cytokines (IL-6 and TNF- α). From this study the peak incidence of PC and BPH subjects was observed at 60-69 years with a mean age of 68.31 ± 7.72 years and 66.5 ± 10.6 years respectively. The observed difference in IL-6 level in the PC and BPH subjects was not statistically significant ($P = .6696$). However, the mean serum TNF- α level (29.6 ± 3.2 pg/ml) was significantly higher in PC subjects when compared to BPH subject levels (25.3 ± 4.7 pg/ml) for TNF- α . This study has shown that PC subjects have higher level TNF- α and as such it can serve as a differential marker for PC.

Keywords: Prostate cancer, interleukin -6, tumor necrosis factor alpha, serum prostate specific antigen.

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1. Introduction

Prostate cancer (PC) is of significant general health problem in men globally. In Nigeria, hospital incidence documented by researchers across the nation is 127/100,000 in Lagos University Teaching Hospital (LUTH), Lagos (Osegbe, 1997), 114/100,000 in University of Port Harcourt Teaching Hospital (UPTH), Port-Harcourt (Eke & Sapira, 2002), 89/100,000 in University of Calabar Teaching Hospital (Ebughe *et al.*, 2016), 11% in University College Hospital (UCH), Ibadan (Ogunbiyi & Shittu, 1999), 16.5% in Kano, northern Nigeria (Muhammed *et al.*, 2008) and 10.1% in Zaria (Oluwole *et al.*, 2015), with the prevalence across the country being between 2-11% (Ogundele & Ikuerowo, 2015).



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The greatest predisposing factor of PC is age (Anyia *et al.*, 2010), mostly occurring in men above 65 years of age (Anis *et al.*, 2013) with a peak incidence at 70-74 years and dropping after 80 years of age (Foster *et al.*, 2011). The diagnosis of PC in males below 40 years is quite rare, but may rise sharply after middle age (Anyia *et al.*, 2010). Other risk factors are diet (Hua *et al.*, 2015), lifestyle (Dennis *et al.*, 2002), genetics (Shen & Abate-Shen, 2010), environmental factors, location, family history (Bott *et al.*, 2005), level of testosterone (Gann *et al.*, 1996), obesity (Calle *et al.*, 2003), smoking and increased intake of alcohol (Schoonen, 2015). Another factor associated with PC is high levels of gonadal steroids, as increased levels of estrogen and testosterone may initiate a prostate-specific inflammatory response. Thus, induced early inflammatory events by sex hormones may create an environment for initiation of PC (Nguyen *et al.*, 2014).

Expressed in the stroma of the prostate gland are IL-6 and TNF- α necessary for regulation of its growth. IL-6 plays important function in hemopoiesis where it promotes the growth and differentiation of eosinophils, differentiation of B-cells into plasma cells and stimulates the secretion of antibodies from these cells, in addition to the induction of acute phase protein reaction. IL-6 is found in prostate stromal cells, while its receptor, IL-6R, is expressed by both stromal and epithelial cells. In chronic inflammation or cancer of the prostate gland, IL-6 and TNF- α are over-expressed. Chronic inflammation activates toll-like receptor nuclear factor kappa B (TLR-NF-Kb) pathway on the inflammatory and prostatic tumor cells; causing the release of IL-6 and TNF- α that induce expression of cyclooxygenase (COX-2) and production of reactive oxygen species (ROS). These ROS induce cell and deoxyribonucleic acid (DNA) damage resulting in mutations and genomic instability that may lead to the initiation, promotion, growth, infiltration and spread of tumor cells (Elkahwaji, 2012).

TNF- α , on the other hand, is released by cells, such as macrophages, that surrounds the prostate gland (Nakashima *et al.*, 1998). Serum TNF- α level is high in subjects with hormone-refractory PC (Muenchen *et al.*, 2000) and is involved in the mediation of both acute and chronic systemic inflammation, by stimulating endothelial cells to produce adhesion molecules and chemokines, which attract neutrophils and macrophages to the injured or infected sites. It also has anti-tumor activity both *in vivo* and *in vitro* (Azria *et al.*, 2003), but functions mainly in immune response regulation (Liping *et al.*, 2014). The concentration of TNF- α in cell determines apoptosis or the division of these cells. High levels trigger signal transduction pathways, such as, nuclear factor kappa B (NF-Kb), MAPK and janus-kinase, leading to the proliferation of tumor cells (Muenchen *et al.*, 2000).

The diagnosis of PC usually occurs incidentally, during post-mortem or during resection of BPH, owing mainly to the absence of definite symptoms. Some common diagnostic methods include; serum prostate specific antigen, prostate biopsy, histological examination of prostate biopsy, trans-rectal ultrasonography and digital resection examination. In Port Harcourt, the diagnosis is usually by combination of digital rectal examination (DRE), serum-prostate specific antigen (serum-PSA) estimation, trans-rectal ultrasonography (TRUS) and histological examination of prostate biopsy. Each method has its limitation that may require further studies to improve the diagnostic value. For instance, serum-PSA may be sensitive, but not specific for cancer. None of its value is confirmatory (Mazzucchelli *et al.*, 2000;

Thompson *et al.*, 2004 and Tan *et al.*, 2008). Since pro-inflammatory cytokines, especially, IL-6 and TNF- α are associated with prostate carcinogenesis (Elkawaji, 2012), their serum levels may correlate with serum PSA levels and therefore, may be important in the detection of PC. It is therefore important to ascertain the serum levels of IL-6 and TNF- α in men with prostate cancer at diagnosis and compare same with the current clinical biomarker, serum prostate-specific antigen (serum-PSA). This would help to determine if an assay of the cytokines will improve the diagnosis of prostate cancer in Port Harcourt.

2. Methodology

This was a non-random, cross sectional, descriptive study carried out in the Urology Clinic, Surgery Department, University of Port Harcourt Teaching Hospital (UPTH), Nigeria. Ethical clearance was obtained from the Ethics Committees of the UPTH and University of Port Harcourt. Authorization was also obtained from the management of a private clinic (Rosyville Clinic and Urology Centre) in Port Harcourt prior to commencement of the study, while informed consent was obtained from each subject before recruitment into the study.

The study population were men (subjects-40 years and above) diagnosed with prostate cancer and BPH at the Urology Clinic in UPTH. A PC prevalence of 4.4% (2-11%) by Ogundele and Ikuerowo (2015) was used to determine the sample size of 80, while a three-part questionnaire of personal biodata, medical history and symptoms and co-morbid infections was used as the instrument for data collection. A vacutainer needle was used to obtain sample which was placed in a plain bottle and stored at -80°C till when needed for analysis. An enzyme-linked immunosorbent assay (ELISA) technique was used for the laboratory analysis of the sample.

Similarly, statistical package for social sciences (SPSS) version 20 was used to analyze the data, taking into consideration both descriptive and inferential statistics, while the results were presented in tables and charts, in tandem with a level of significance of ≤ 0.05 and 95% confidence interval.

3. Results

Ninety - eight (98) subjects were evaluated for prostate disease. Eighty (80) with features of PC were further examined using histological technique on prostate biopsies; sixty (60) had PC while twenty (20) had BPH.

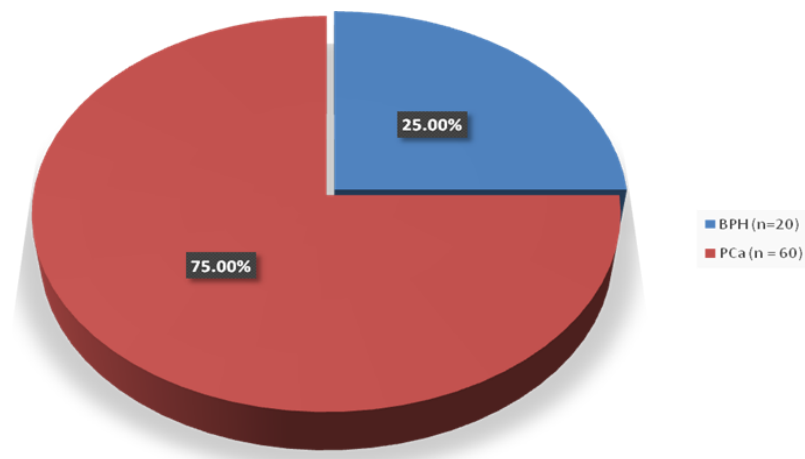


Figure 1. Distribution of BPH and PC in study subjects

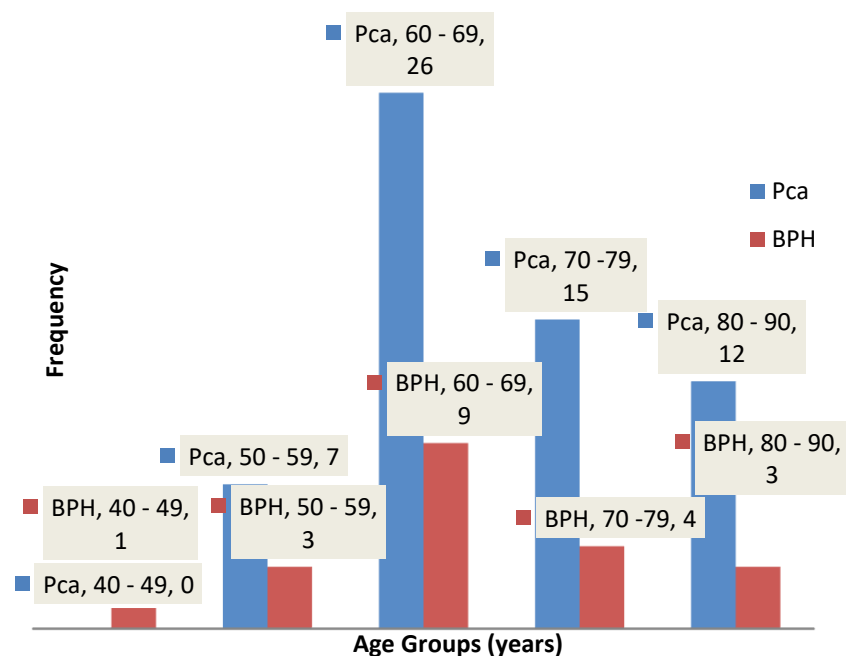


Figure 2. Distribution of study subjects by age groups.

Figure 2 gives a description of the age distribution of the subjects. Prostate Cancer is not common below the age of 50years. Its incidence increases with age. Seven (11.67%) of the subjects were in the age group of 50-59 years. The peak incidence of PC was observed in the age group of 60-69years (26) accounting for 43.3%, while there was a decline in age group 70-79years with 15(25%) and 12 (20%) in age 80-90years. The mean age was 68.31 ± 7.72 years.

Table 1. Serum mean IL-6 and TNF- α levels and standard deviation in PC and BPH subjects

Variable	PC	BPH	T-Test (p-value)
IL-6(pg/ml)	8.7 $\pm$ 3.0	8.4 $\pm$ 1.2	0.6696**
TNF- α (pg/ml)	29.6 $\pm$ 3.2	25.3 $\pm$ 4.7	0.0001*

*Observed difference is statistically significant ($p < 0.05$); **Observed difference is statistically not significant ($p > 0.05$).

From table 1 above, the serum mean level for TNF- α (25.3 ± 4.7) pg/ml and IL-6 (8.4 ± 1.2) pg/ml were both lower in BPH than in the study group. This observed difference for TNF- α was statistically significant ($P = 0.0001$). However, for IL-6 the observed difference was not statistically significant ($p = 0.6696$).

Table 2. Serum mean levels and ranges of IL-6 and TNF- α with standard deviation in different age groups of the prostate cancer subjects

Age group (years)	IL-6(pg/ml)	TNF- α (pg/ml)
50 – 59	13.9 $\pm$ 5.6 (3.9-64.2)	19.7 $\pm$ 8.1 (11.4-27.1)
60 – 69	10.8 $\pm$ 5.4(3.9-61.9)	27.4 $\pm$ 10.1(14.6-51.4)
70 – 79	11.2 $\pm$ 3.0(4.6-44.6)	22.7 $\pm$ 9.5(12.9-41.5)
80 – 90	11.9 $\pm$ 16.7(3.2-63.6)	17.8 $\pm$ 8.6(10.7-37)

Table 2 above shows that age did not affect the levels of these parameters in the PC subjects.

Table 3. Serum mean levels and ranges of IL-6 and TNF- α with standard deviation in different age groups of the BPH subjects

Age group (years)	IL-6 (pg/ml)	TNF- α (pg/ml)
40 – 49	5.1	16.3
50 – 59	4.76 $\pm$ 6.6(3.9-5.9)	18.76 $\pm$ 8.3 (11.8-27.1)
60 – 69	12.8 $\pm$ 4.4 (3.9-61.9)	29.1 $\pm$ 11.1 (14.6-51.4)
70 – 79	4.6 $\pm$ 3.6 (4.6-6.1)	35.1 $\pm$ 19.5 (12.9-41.5)
80 – 90	57.6 $\pm$ 6.7 (4.2-163.6)	15.5 $\pm$ 6.3 (10.7-20.1)

Table 3 shows that age did not affect the levels of these parameters in the BPH subjects

4. Discussion

PC is the most commonly detected malignancy in men above the age of 40 years with very high morbidity and mortality rate worldwide. The high incidence of PC has necessitated the need for clinical biomarkers to enable quick detection of the onset and advancement of the disease. Emerging evidence has shown the involvement of pro-inflammatory cytokines in PC initiation and progression (Bardia *et al.*, 2009; Tse *et al.*, 2012).

Serum levels of IL-6 and TNF- α were assayed in PC and the BPH subjects. The PC subjects had higher levels of both variables than the BPH subjects. The increased levels of IL-6 and TNF- α supports the suggestion that inflammation may play a significant role in the pathogenesis of PC. The raised levels of IL-6 and TNF- α observed in this study indicate the presence of chronic inflammation in both the PC and BPH subjects. The TNF- α and IL-6 levels in PC and BPH subjects were different, and the differences were statistically significant ($p < 0.0001$) except for IL-6. Cohen *et al.* (2005) reported chronic and or acute glandular inflammation and thus, confirms the role of inflammation observed in this study. Epithelial cells of the prostate gland, during inflammation, produce pro-inflammatory cytokines (Hobisch *et al.* (2000). The increased mean levels of IL-6 and TNF- α observed in the subjects with PC in this

study are similar to what was reported by Tazaki *et al.* (2011) and that of Tse *et al.* (2012).

This study showed that age is a strong predisposing factor to the aetiology of PC. Low incidence rate was recorded in the middle age. The highest prevalence was recorded in the age group 60-69years with a mean age of 68years. This age peak in incidence is similar to the observations of Ibrahim *et al.* (2015) and Adeloye *et al.* (2016) who both observed a peak incidence at age group 60-69years. The observation from this study also agrees with that of Anis *et al.* (2013) with peak incidence at 65 years, but at variance with the findings of Foster *et al.* (2011) with peak incidence at 70-74years. There was a steady increase in the incidence of PC from 60-79years and a decline after age 79. This finding also agrees with the observations of Mohammed *et al.* (2005), Foster *et al.* (2011) and Obiorah and Nwosu (2011). The reason for this peak incidence at age group 60-69 years may be due to decline in immune system function, especially, from the middle age of 50 years, which according to Hakim *et al.* (2004) is due to reduction in the function of the haemopoietic stem cell (HSC) that give rise to all other cells of the immune system.

5. Conclusion

This study has demonstrated that both IL-6 and TNF- α were present in PC and BPH subjects probably due to inflammation, however, TNF- α level significantly raised in PC subject which suggests that TNF- α may be a differential marker in PC.

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